REMARKS

In the Action, the Examiner rejected claims 1-17 and 22-28 under 35 USC §112, second paragraph, as assertedly being indefinite and rejected claims 1-11, 13-16, 22-26 and 28 under 35 USC §112, first paragraph, as assertedly lacking enablement. The Examiner also rejected claims 1-7, 12-17, 24 and 27 under 35 USC §103(a) as assertedly obvious in view of Meinhardt (ref C1 on Applicants IDS, hereinafter "Meinhardt"), Butler et al. (Yeast 7:617-25, 1991) (hereinafter "Butler"), further in view of U.S. Patent 6,410,271 (hereinafter "the 271 patent"), further rejected claims 22 and 23 as obvious in view of Meinhardt, Butler, the '271 patent and Monschau (Applied Envtl Microbiol 64: 4283-90, 1998) (hereinafter "Monschau"), and rejected claim 28 as obvious in view of Meinhardt, Butler, the '271 patent and Jirholt (Gene 215:471-76, 1998) (hereinafter "Jirholt"). Reconsideration is requested in light of the following amendments and remarks.

I. Objections to the Specification

The Examiner objected to the specification as lacking sequence identifiers for sequences in the specification. Applicants submit that the specification has been amended to include sequence identifiers where necessary.

With respect to the amendment at page 10, line 5, the sequence identifiers for CDRf_40 and CDRf_60 were incorrectly listed as SEQ ID NO: 2 and SEQ ID NO: 3, respectively. In the Sequence Listing they are referred to in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The change to the specification now correctly identifies the sequence identifiers.

The Examiner asserted that a sequence identifier is necessary for the string of amino acids "KLGT" at page 4, lines 35-36. Applicants submit that the term "KLGT" at page 4 is the same as that at page 12, lines 25-26 of the specification, which states that "the γ-toxin lacking the signal peptide (referred to as KLGT)." The paragraph at page 4, line 34, has been amended to clarify that "KLGT" does not refer to a string of amino acids but is a notation for K. Lactis gamma toxin lacking a signal peptide.

The amendments include no new matter.

II. Support for the Amendments to the Claims

Support for the amendment to the claims is found throughout the specification. For example, claim 1 has been amended to include reference to yeast cells, which are described throughout the specification and the Examples section. Support for new claims 31-33 is found in original claims 2 and 15. Claim 8 has been amended to correct a typographical error. Claim 11 has been amended to correct antecedent basis. The amendments include no new matter.

III. The rejection of claims 1-17 and 22-28 under 35 USC §112, second paragraph, as assertedly indefinite should be withdrawn

The Examiner variously rejected claims 1-17 and 22-28 under 35 USC §112, second paragraph, as assertedly indefinite as follows.

Claim 1 was rejected as assertedly being indefinite for recitation of the terms "suitable" e.g., suitable cells and suitable conditions. Suitable as used in the present context and in standard usage means "appropriate for." It is clear to the skilled person that "suitable cells" useful in the present methods are capable of allowing homologous recombination (see page 3, lines 3-4) and are sensitive to the gamma toxin (page 7, lines 15-31). For the skilled person it is also clear that "suitable conditions" refers to conditions which allow the chosen cells to grow, multiply, perform homologous recombination, and express the gamma toxin when no homologous recombination takes place. Page 3, lines 18-24, state "under suitable conditions allowing the selection of host cells in which said DNA sequence in the target vector ... has been replaced by said donor sequence by means of homologous recombination thereby abolishing expression of said γ-subunit of a K. lactis killer toxin," thereby describing suitable conditions for carrying out the method of the invention. One of ordinary skill would readily be able to determine suitable cells or conditions based on the Examples in the specification and the knowledge in the art. As such, the claim is clear as written and the rejection of the claim as indefinite should be withdrawn.

Claims 2 and 15 were rejected as assertedly being indefinite for recitation of the phrase "preferably." Claims 2 and 15 have been amended to remove reference to "preferably," thereby obviating the objection.

Claims 6, 7, 14 and 24 were rejected as assertedly being indefinite for recitation of the phrase "unique recognition site." The Examiner alleged it is unclear what makes a recognition site unique. The term "unique" is used in the sense of a single restriction site, i.e., that a specific restriction enzyme will cut only once in a given DNA sequence. This term is well-known to the skilled person. For example, in the embodiment described at page 5, lines 3-8, of the specification, the target vector is introduced into the host cells in linearized form. The linearization of the target vector is achieved by cutting the DNA sequence with a restriction enzyme which cuts only once in the entire DNA sequence of the target vector, thus the enzyme has a unique recognition site in the entire DNA sequence of the target vector. The example beginning at page 13, line 19 (to page 15, line 23) exemplifies this use when the target DNA is linearized and integrated at the PacI restriction site and the gamma toxin is removed by site-directed homologous recombination. This unique restriction site allows for efficient integration of the target sequence into the desired site, whereas multiple sites having the same restriction sequence would not ensure proper recombination. Thus, the term is understood by one of ordinary skill in the art and the claim is clear as written.

Claim 8 was rejected as assertedly being indefinite for recitation of the phrase "said second DNA sequence." Applicants submit that is was a typographical error and Claim 8 has been corrected to include reference to a "first" DNA sequence. Reading claim 8 in combination with claims 9 and 10, it is clear to the skilled person that the embodiment described at page 13, line 22, to page 14, line 7, and in Figure 3, e.g., wherein the DNA is inserted into a first DNA sequence, is to be covered by claim 8.

Amendment to claim 8 clarifies the objection to claim 9.

Claim 11 was rejected as assertedly being indefinite for recitation of the phrase "the signal peptide," alleging there is insufficient antecedent basis for this phrase in the claim. Claim 11 has been amended to refer to "a signal peptide," thereby obviating the rejection However, one of ordinary skill would recognize that the γ subunit is typically expressed with a signal peptide which is therefore endogenous to the toxin. It is clear to the skilled person that the γ subunit can optionally be expressed in a recombinant system with or without said signal peptide. The definition of the term "DNA sequence encoding a γ subunit of the K. lactis killer toxin" at page 8, lines 10-15, of the specification comprises all DNA

sequence variations which encode a functional γ subunit, i.e., a subunit which leads to loss of viability in sensitive cells. Therefore, it is clear that the γ subunit can be expressed with or without a signal peptide.

For the foregoing reasons, the rejection of the claims under 35 USC §112, second paragraph, should be withdrawn.

IV. The rejection of claims 1-11, 13-16, 22-26 and 28 under 35 USC §112, first paragraph, as assertedly lacking enablement should be withdrawn

The Examiner rejected claims 1-11, 13-16, 22-26 and 28 under 35 USC §112, first paragraph, as assertedly lacking enablement in the specification for use of any cell in the method of the invention. The Examiner acknowledges, however, that Applicants have enabled use of yeast cells in the method (page 5 of the Action).

In order to expedite prosecution, the claims have been amended to recite that the host cells are yeast cells, thereby obviating the rejection.

V. The rejection of claims 1-7, 12-17, 24 and 27 under 35 USC §103(a) as assertedly obvious in view of Meinhardt, Butler and US 6,410,271 should be withdrawn

The Examiner rejected claims 1-7, 12-17, 24 and 27 under 35 USC §103(a) as assertedly obvious in view of Meinhardt and Butler, further in view of the '271 patent, alleging that one of ordinary skill would readily be able to make a randomized gene library having the K. lactis gamma subunit based on the teachings in the art. The Examiner asserts that Meinhardt allegedly teaches a plasmid comprising the K. lactis killer toxin gene, that Butler allegedly teaches the gamma subunit gene, and that the '271 patent allegedly teaches methods for generating libraries of expression vectors encoding fusion proteins, and therefore one of ordinary skill would combine these teachings to arrive at the present invention. Applicants respectfully disagree.

The methods of the invention are directed to a randomized homologous recombination library wherein the gamma toxin is used as a negative selection marker. Through use of this marker, successful homologous recombination can be directly evidenced by the growth of the host cells. Only if the donor DNA replaces the target sequence, i.e., the gamma toxin, will the cells grow, and thereby there is negative selection against non-

homologous recombination that helps ensure correct integration of the donor sequence into the genome. Further, the vector background, i.e., presence of the circular vector, is low. The presence of the circular vector is linked to the presence of the gamma toxin which leads to growth arrest of the corresponding host cells.

To establish a *prima facie* case of obviousness, the examiner must show that all the elements of the claim are taught or suggested in the prior art (MPEP 2143.03 and Federal Register Examination Guidelines for Determining Obviousness, Section III.A.1, Fed Reg., Vol 72, No. 195, 2007), and if prior art elements are described in the art, the combination of elements must yield predictable results to render a claimed invention obvious. Further, it should be demonstrated that the prior art reference(s) provide a teaching, suggestion or motivation to combine the references, and/or there is a reasonable expectation of success (MPEP 2142 and Federal Register Examination Guidelines for Determining Obviousness, Section III.G, Fed Reg., Vol 72, No. 195, 2007). The court in KSR v Teleflex (127 S.Ct. 1727 (2007)) further stated that mere conclusory statements are not sufficient to draw a conclusion of obviousness, but that there must be some articulated reasoning with some rational underpinning to support a legal conclusion of obviousness. See Fed Reg., Vol 72, No. 195, Pages 57529 and KSR v Teleflex, 127 S.Ct. 1727.

In the present case, the Examiner has failed to establish a *prima facie* case of obviousness since not all of the elements of the claims are disclosed in the cited art. None of the art, taken alone or in combination, discloses use of the gamma toxin gene as a negative selection marker as recited in the claims.

Meinhardt discloses use of a target vector having the general linear plasmid pGKL1 of K. lactis coding for the entire killer toxin gene. Meinhardt discloses a donor DNA sequence flanked by a DNA sequence which is homologous to the target sequence in pGLK1 (Figure 2 of Meinhardt). Upon homologous recombination in a suitable cell, i.e. K. lactis, the ORF2 of the killer toxin gene is replaced by two marker genes, LEU2 and Aph, and in the resulting vector, pWKL1, the donor genes LEU2 and Aph and the gamma toxin gene are present. Thus, it is the selection markers which are the donor sequences and integrate into the target vector and not the target DNA sequence as in the present method. The method of Meinhardt is not typically useful for generating a randomized gene library (as required by the

claims) given the use of the selection markers as donor sequences. Further, Meinhardt uses cells that are resistant to the gamma toxin gene effects, and does not suggest use of cells susceptible to gamma toxin that are suitable for use in the present invention. Meinhardt neither discloses nor suggests use of the gamma toxin as a negative selection marker such that excision of this gene results in selection for positive clones, and wherein the gamma toxin cannot be expressed with the donor DNA.

Butler discloses that target cells (S. cerevisiae) that have been transformed with a plasmid carrying the gamma gene (pGKL1 ORF4) are unable to form colonies, and teaches that only the gamma subunit is essential for the inhibitor action of the K. lactis killer toxin (p 617, 2nd col.). As such, Butler neither discloses nor suggests that the gamma toxin is useful as a negative selection marker which is removed upon homologous recombination with donor DNA.

The '271 patent relates to the production of expression libraries and generation of fusion proteins using homologous recombination in yeast. The '271 patent discloses only the use of positive selection markers, such as nutritional reporter genes, for carrying out homologous recombination (col. 40, lines 36-48). The '271 patent neither discloses nor suggests that a negative selection system as taught in the present application, using the gamma toxin gene as a negative selection marker, would be useful for constructing a randomized recombination library.

As noted above, none of the cited art teaches that the gamma toxin gene is useful as a negative selection marker such that excision of the gamma toxin gene signifies successful homologous recombination of the donor and target DNA. Further, none of the art discloses use of a cell line susceptible to gamma toxin effects for homologous recombination when using the gamma toxin gene as a negative selection marker.

Because none of the art discloses use of the K. lactis gamma toxin gene as a negative selection marker in a homologous recombination event, the Examiner has failed to establish a *prima facie* case of obviousness and the rejection of claims 1-7, 12-17, 24 and 27 under 35 USC §103(a) as obvious in view of Meinhardt, Butler and the '271 patent should be withdrawn.

The Examiner further rejected claims 22 and 23 as obvious in view of Meinhardt, Butler, the '271 patent and Monschau. Monschau describes use of the TEF promoter from A. gossypii, and neither discloses nor suggest use of the gamma toxin gene as a negative selection marker.

As stated above, the combination of Butler, Meinhardt and the '271 patent does not render obvious any of claims 1-7, 12-17, 24 and 27 since none of the references describe use of the gamma toxin gene as a negative selection marker. Monschau does not remedy this deficiency. Thus, the rejection of claims 22 and 23 under 35 USC §103(a) should be withdrawn.

The Examiner further rejected claim 28 as obvious in view of Meinhardt, Butler, the '271 patent and Jirholt. Jirholt discloses a recombination library encoding CDR sequences, but neither discloses nor suggests use of the gamma toxin gene as a negative selection marker.

As stated above, the combination of Butler, Meinhardt and the '271 patent does not render obvious any of claims 1-7, 12-17, 24 or 27 since none of the references describe use of the gamma toxin gene as a negative selection marker. Jirholt does not remedy this deficiency. Thus, the rejection of claim 28 under 35 USC §103(a) should be withdrawn.

VI. Conclusion

Applicants submit that the application is in condition for allowance and respectfully request notice of the same.

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Respectfully submitted,

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